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[54] **DNA FRAGMENT COMPRISING A GENE ENCODING ETHYLENE FORMING ENZYME OF BACTERIA AND THE USE THEREOF**

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[30] **Foreign Application Priority Data**

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[51] Int. Cl.⁶ C12N 1/20; C12N 15/63; C07H 21/04

[52] U.S. Cl. 435/252.33; 435/320.1; 536/23.2

[58] Field of Search 435/252.3, 167, 435/252.33, 320.1; 536/23.2

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,877,730 10/1989 Urushizaki et al. 435/132

OTHER PUBLICATIONS

Nagahama, K. et al. (1992) "Classification of ethylene-producing bacteria in terms of biosynthetic pathways to ethylene" *J. Ferment. Bioeng.* 73(1):1-5.

Fukuda et al., *Biochemical and Biophysical Research Communications*, vol. 188, No. 2, Oct. 30, 1992, 826-832.

Nagahama et al., *Journal of General Microbiology*, vol. 137, 1991, 2281-2286.

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[57] **ABSTRACT**

Bacteria such as *E. coli* which are easy in manipulation are transformed by the gene encoding a ethylene-forming enzyme, and ethylene is formed by the transformants.

7 Claims, 2 Drawing Sheets

10 20 30 40 50
 GCGACCGCGTGTGTCAGCAACGACAATCCTTACTCGGAGTCGCTGTTGAGGACACTGAAGT
 60 70 80 90 100 110
 ACTGCCCGCAATGGCCGAGGATGGGTTTGCCAGTCTTGACGCGGCACGCTCTGGGTGAG
 120 130 140 150 160 170
 GGATTTTCATGCGTTGGTACAACAATGATCACCGGCACAGCCGAATCCGCTTCGTGACACC
 180 190 200 210 220 230
 GGCTGAGCGGCATCGAGGGCTGGATCATCAGATCCTGGCCAAGCGACATGAGCTGTACGA
 240 250 260 270 280 290
 GCTAGCCAGAGAGAAAAGGCCGGAGCGGTGGTCGAGGGAGACACGCAACTGGGAACCGAT
 300 310 320 330 340 350
 CGGCACCGTGCTGTTAAACCCGGATCGAGAGCAAGACGTTGAGAAAAAAGCAGCATAGTT
 360 370 380 390 400 410
 AGACGGTTGACGCGACAACCTACCTTGAAAAACGCCGGCAGGGACGCTCATGATTCATGCT
 420 430 440 450 460 470
 CCAAGCAGGTGGGGTGTITTTCCGTCGCTTGGACTTTGTTCTCCCGACGTCGTTTGAAT
 480 490 500 510 520 530
 GAGCATCCGTCCTTTATATGGATAAAGAAGAGACTAGCATGACCAACCTACAGACTTTC
 M T N L Q T F
 540 550 560 570 580 590
 GAGTTGCCTACCGAGGTAACCGGCTGCGCCGCCGATATCTCATTGGGAAGGGCGCTGATC
 E L P T E V T G C A A D I S L G R A L I
 600 610 620 630 640 650
 CAAGCCTGGCAAAAAGATGGCATTTTTTCAGATCAAGACCGATAGTGAGCAGGATCGCAA
 Q A W Q K D G I F Q I K T D S E Q D R K
 660 670 680 690 700 710
 ACGCAGGAAGCAATGGCTGCTAGCAAGCAGTTTTGCAAGGAACCGCTGACTTTTAAGAGT
 T Q E A M A A S K Q F C K E P L T F K S
 720 730 740 750 760 770
 AGCTGCGTTAGCGATCTGACCTACAGCGGCTATGTTGCGTCAGGCGAGGAAGTCACAGCT
 S C V S D L T Y S G Y V A S G E E V T A
 780 790 800 810 820 830
 GGTAACCTGATTTCCCTGAAATCTTCACTGTCTGCAAGGACTTGTGCGGTAGGCGATCAG
 G K P D F P E I F T V C K D L S V G D Q
 840 850 860 870 880 890
 CGTGTAAGCCGGCTGGCCTTGCCATGGTCCGGTGCCATGGCCAAATAACACCTATCAG
 R V K A G W P C H G P V P W P N N T Y Q
 900 910 920 930 940 950
 AAAAGCATGAAGACCTTCATGGAAGAGCTGGGTTTAGCGGGCGAACGGTTGCTCAAACCTG
 K S M K T F M E E L G L A G E R L L K L

FIG. 1

960 970 980 990 1000 1010
 ACAGCGCTCGGCTTTGAACTACCCATCAACACGTTACCGACTTAACTCGCGATGGTTGG
 T A L G F E L P I N T F T D L T R D G W

 1020 1030 1040 1050 1060 1070
 CACCACATGCGTGTATTACGCTTCCCGCCCCAAACATCCACGCTGTCCCGTGAATTGGT
 H H M R V L R F P P Q T S T L S R G I G

 1080 1090 1100 1110 1120 1130
 GCGCACACTGACTATGGGTTGTTGGTAATTGCCGCTCAGGACGATGTTGGTGGCTTATAT
 A H T D Y G L L V I A A Q D D V G G L Y

 1140 1150 1160 1170 1180 1190
 ATTCGCCCTCCAGTCGAGGGAGAGAAGCGTAATCGTAACTGGTTGCCTGGTGAGAGCTCA
 I R P P V E G E K R N R N W L P G E S S

 1200 1210 1220 1230 1240 1250
 GCAGGCATGTTTGAGCACGATGAACCTTGGACCTTCGTGACGCCACCCAGGCGTGTGG
 A G M F E H D E P W T F V T P T P G V W

 1260 1274 1280 1290 1300 1310
 ACAGTTTTCCAGGTGATATCTTGCAGTTCATGACCGGCGGCCAGCTGCTTCCACTCCG
 T V F P G D I L Q F M T G G Q L L S T P

 1320 1330 1340 1350 1360 1370
 CACAAGGTTAAGCTCAATACCCGCGAACGTTTCGCCTGCGCTTATTTTCATGAGCCTAAT
 H K V K L N T R E R F A C A Y F H E P N

 1380 1390 1400 1410 1420 1430
 TTTGAAGCATCCGCCTATCCGTTGTTTCGAGCCCAGCGCCAATGAGCGTATTCATTATGGT
 F E A S A Y P L F E P S A N E R I H Y G

 1440 1450 1460 1470 1480 1490
 GAGCACTTTACCAACATGTTTATGCGTTGCTATCCAGATCGGATCACCACCCAGAGGATC
 E H F T N M F M R C Y P D R I T T Q R I

 1500 1510 1520 1530 1540 1550
 AACAAGGAGAATCGCCTGGCGCACTTGGAGGACTTGAAGAAGTATTCGGACACCCGCGCG
 N K E N R L A H L E D L K K Y S D T R A

 1560 1570 1580 1598 1600 1610
 ACAGGCTCATGAGTCGACACCCTGCCCGGTGCTGCCGACAGGGGCCTTATCGTTACTGG
 T G S

 1620 1630 1640 1650 1660 1670
 TGACTAATAATTGGCATATCAATGTCCACTCAGCACCCAGATCTTATGATCTGGGTGCTG

 1680 1690 1700 1710 1720 1730
 AGTGGAGCAATGTAACCATTATGCTGAGCGTTCATGCATAGGAATTTCAATAATTCCTAT

 1740 1750 1760 1770 1780 1790
 ACAAGGCAATCCGCCGAAAAAGGTCCCCTCGGCGTGATGCCAACGTGGCGTCGATGTCCG

 1800
 CAAAAAGCTT

FIG. 2

1

**DNA FRAGMENT COMPRISING A GENE
ENCODING ETHYLENE FORMING
ENZYME OF BACTERIA AND THE USE
THEREOF**

TECHNICAL FIELD

This invention relates to a DNA fragment comprising a gene encoding an ethylene-forming enzyme of bacteria, a vector including the DNA fragment, transformants transformed with the vector, and a method for producing ethylene using the transformants.

BACKGROUND ART

While ethylene is produced from crude petroleum or natural gas, it has long been known that ethylene could also be formed by plants and microorganisms.

Chou et al. reported that α -ketoglutaric acid(α -KG) or L-glutamic acid(Glu) is a precursor of ethylene in *Penicillium digitatum*, a species of fungi (Arch. Biochem. Biophys., 157, 73, 1973), and Goto et al. reported that α -KG serves as a substrate in the ethylene forming system in cell-free extracts of *Pseudomonas syringae* which is one of the species of pathogenic bacteria for plants (Plant Cell Physiol., 28, 405, 1987).

Primrose et al. reported that, in the ethylene-forming system in cell-free extracts of *Escherichia coli*, 2-keto-4-methylthiobutyric acid(KMBA) which is a metabolic intermediate of L-methionine(Met) is a precursor of ethylene biosynthesis (J. Gen. Microbiol., 98, 519, 1977). In all these reports, however, the true substrates and biosynthetic pathway of ethylene-forming reaction were not established because materials such as cultured cells of bacteria or their cell free extracts, which are supposed to contain a lot of impurities, were used in the experiments.

To make clear the pathway of the ethylene biosynthesis caused by the bacteria and the ethylene-forming enzyme reaction, the present inventors purified the enzyme catalyzing the ethylene formation to electrophoretically homogeneous state. The results revealed that the ethylene-forming reaction via KMBA is really radical reactions in which active oxygen is concerned (Fukuda et al., FEMS Microbiol. Lett., 60, 107, 1989). We have also investigated the ethylene-forming enzyme of *Penicillium digitatum* IFO 9372 via a α -KG and its enzymatic reaction (Fukuda et al., FEMS Microbiol. Lett., 59, 1989), and the ethylene-forming enzyme of *Pseudomonas syringae* pv. phaseolicola PK2 via α -KG and its characteristics (Nagahama, Fukuda et al., J. Gen. Microbiol., 137, 2228, 1991).

Although the enzyme catalyzing the ethylene biosynthesis by bacteria was identified as well as the characteristics of the enzyme, by the present inventors as described above, the ability of ethylene formation in these ethylene-forming bacteria was not adequately sufficient.

For the purpose of fundamental breeding improvement of the ethylene-forming bacteria through a gene manipulation technique, *Pseudomonas syringae* pv. phaseolicola PK2 was selected for the object and its DNA sequence encoding the ethylene-forming enzyme was analyzed, thereby completing this invention.

DISCLOSURE OF INVENTION

To achieve the above described object, this invention is characterized in that a gene encoding an amino acid sequence represented by:

2

Sea ID No: 1 Met Thr Asn Leu Gln Thr Phe Glu Leu Pro Thr Glu Val Thr Gly Cys Ala Ala Asp Ile Ser Leu Gly Arg Ala Leu Ile Gln Ala Trp Gln Lys Asp Gly Ile Phe Gln Ile Lys Thr Asp Ser Glu Gln Asp Arg Lys Thr Gln Glu Ala Met Ala Ala Ser Lys Gln Phe Cys Lys Glu Pro Leu Thr Phe Lys Ser Ser Cys Val Ser Asp Leu Thr Tyr Ser Gly Tyr Val Ala Ser Gly Glu Glu Val Thr Ala Gly Lys Pro Asp Phe Pro Glu Ile Phe Thr Val Cys Lys Asp Leu Ser Val Gly Asp Gln Arg Val Lys Ala Gly Trp Pro Cys His Gly Pro Val Pro Trp Pro Asn Asn Thr Tyr Gln Lys Ser Met Lys Thr Phe Met Glu Glu Leu Gly Leu Ala Gly Glu Arg Leu Leu Lys Leu Thr Ala Leu Gly Phe Glu Leu Pro Ile Asn Thr Phe Thr Asp Leu Thr Arg Asp Gly Trp His His Met Arg Val Leu Arg Phe Pro Pro Gln Thr Ser Thr Leu Ser Arg Gly Ile Gly Ala His Thr Asp Tyr Gly Leu Leu Val Ile Ala Ala Gln Asp Asp Val Gly Gly Leu Tyr Ile Arg Pro Pro Val Glu Gly Glu Lys Arg Asn Arg Asn Trp Leu Pro Gly Glu Ser Ser Ala Gly Met Phe Glu His Asp Glu Pro Trp Thr Phe Val Thr Pro Thr Pro Gly Val Trp Thr Val Phe Pro Gly Asp Ile Leu Gln Phe Met Thr Gly Gly Gln Leu Leu Ser Thr Pro His Lys Val Lys Leu Asn Thr Arg Glu Arg Phe Ala Cys Ala Tyr Phe His Glu Pro Asn Phe Glu Ala Ser Ala Tyr Pro Leu Phe Glu Pro Ser Ala Asn Glu Arg Ile His Tyr Gly Glu His Phe Thr Asn Met Phe Met Arg Cys Tyr Pro Asp Arg Ile Thr Thr Gln Arg Ile Asn Lys Glu Asn Arg Leu Ala His Leu Glu Asp Leu Lys Lys Tyr Ser Asp Thr Arg Ala Thr Gly Ser

is integrated into bacteria.

DNA encoding the ethylene-forming enzyme of the present invention can be obtained, for example, by the procedures comprising:

preparing a probe with an amino acid sequence coding the ethylene-forming enzyme from *Pseudomonas syringae*; hybridizing this probe with an indigenous plasmid by Southern method to find that a gene for the ethylene-forming enzyme is encoded in the indigenous plasmid DNA; constructing a gene library after transforming *Escherichia coli* (*E. coli*) using restriction enzyme Hind III fragments of this indigenous plasmid DNA and pUC19 as a vector; and screening a positive clone *E. coli* JM109 (pEFE01) from the library by a hybridization using Southern method.

Hind III fragments of about 2.5 kbp from *Pseudomonas syringae* were inserted into the plasmid pUC19 in the cell of this positive clone *E. coli* JMO109(pEFE01), and an ethylene-forming activity was found in this positive clone *E. coli* JM109(pEFE01). This positive clone *E. coli* JM109(pEFE01) also expressed a protein that could not be distinguished from the ethylene-forming enzyme protein of *Pseudomonas syringae* as detected by Western blotting method using an antibody for the ethylene forming enzyme.

Deletion mutant strains having various sizes of plasmids were prepared by digesting the inserted 2.5kbp Hind III fragments. The smallest pEFE01 derivative was obtained from the mutants keeping ethylene-forming activity. The size of the smallest fragment was about 1.5 kbp.

BRIEF DESCRIPTION OF DRAWOMGS

FIG. 1 shows a base sequence of the gene of the ethylene-forming enzyme after cloning and the first half portion of the amino acid sequence translated therefrom.

FIG. 1 shows a base sequence of the gene of the ethylene-forming enzyme after cloning and the latter half portion of the amino acid sequence translated therefrom.

BEST MODE FOR CARRYING OUT THE
INVENTION

EXAMPLES

Chromosomal DNA of *Pseudomonas syringae* was extracted with phenol after lysis of cell wall with lysozyme and sodium dodecyl sulfate (SDS), wherein proteins were removed by denaturation and DNA was recovered from the supernatant by ethanol precipitation. The plasmid DNA of *Pseudomonas syringae* was extracted by an alkali extraction method.

Extracted DNA was digested with a restriction enzyme Hind III and the digested fragments were subjected to electrophoresis using 0.7% of agarose gel. DNA in the gel after the electrophoresis were transferred to a Nylon membrane by a capillary method, bound onto the membrane by a dry-heat treatment and were subjected to Southern hybridization.

Since the structural gene of the ethylene-forming enzyme (EFE) seemed to be located on the plasmid after the hybridization, the plasmid DNA fragments digested with Hind III are ligated with the *E. coli* vector (pUC19) subjected to an alkali phosphatase treatment, thereby obtaining the chimera plasmid. This chimera plasmid was mixed with competent cells of *Escherichia coli* (*E. coli* JM109) prepared by a calcium chloride method and, after allowing them to stand in an ice bath for an hour, the chimera plasmid was incorporated into the cell with a heat shock, followed by centrifugation, thereby transforming *E. coli* JM108. This *E. coli* JM109 after the transformation was seeded in 2xYT culture medium consisting of 16g/liter of Bactotrypton, 10g/liter of Bactoyeast extract and 5g/liter of sodium chloride. After the cultivation for 1.5 hours, *E. coli* JM109 was separated by centrifugation and inoculated in a selected culture medium.

Colonies having the chimera plasmid expressed in the selected culture medium were screened and these colonies were cultivated in LB culture medium consisting of 10g/liter of Bactotrypton, 5g/liter of Bactoyeast extract and 10g/liter of sodium chloride supplemented with 5 µg/ml of thiamine hydrochloride and 50 µg/ml of ampicillin sodium salt. The plasmid DNA (chimera pUC19) was extracted with an aqueous alkaline, purified by removing RNA and, after treating with Hind III, it was subjected to electrophoresis using 1% of agarose gel. The DNA after electrophoresis was denatured with an aqueous alkaline solution followed by neutralization and blotted on a nylon membrane, and then *E.*

coli which retained the gene of the ethylene-forming enzyme was screened by hybridization.

When *E. coli* JM109(pEFE01) was cultivated in the above described LB culture medium supplemented with 5g/ml of L-glutamic acid, 5 µg/ml of thiamine hydrochloride and 50 µg/ml of ampicillin sodium salt, the ethylene-forming activity was 230nl/ml of culture medium/hr. In the above described *E. coli* JM109 (a disclosed strain well known in the art) used as a host, on the other hand, any ethylene-forming activity was not detected and its ethylene-forming rate was 0nl/ml of culture medium/hr. The term "ethylene-forming activity" used herein refers to an in vitro ethylene-forming ability in a cell-free extract prepared from the cells, and the term "ethylene-forming rate" refers to an in vivo ethylene-forming ability in the culture medium. These characteristics could be detected by conventional methods described, for example, in the above described reference (Nagahama, Fukuda et al., J. Gen. Microbiol., 37, 2281, 1991).

The transformant *Escherichia coli* JM109(pEFE01) used in this invention was deposited to National Institute of Bioscience and Human-Technology, 1-3, Higashi-chome, Tsukuba City, Japan, under the registration number of FERM P-1361 dated Sep. 16, 1992.

It was made clear by Western blotting that an identical protein with the ethylene-forming enzyme of *Pseudomonas syringae* was also present in *E. coli* JM109(pEFE01). Deletion mutant having various size of plasmids were prepared by digesting the inserted 2.5kbp Hind III fragment from its terminal. The smallest pEFE01 transformant retaining the ethylene-forming activity was about 1.5 kbp. In addition, neither site for Hind III nor sites for other restriction enzymes PstI, Dra I, EcoR I and BamH I were present in this fragment.

The base sequence and amino acid sequence of the gene of the ethylene forming-enzyme incorporated in 2.5 kbp Hind III fragment as determined by a dideoxy method are shown in FIG. 1 and FIG. 2.

Industrial Applicability

According to this invention, bacteria having high ethylene-forming activity can be obtained by transforming bacteria such as *E. coli* which is safe and easy in manipulation.

Moreover, ethylene can be produced with high efficiency by utilizing these transformed bacteria.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 4

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 350 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

M e t T h r A s n L e u G l n T h r P h e G l u L e u P r o T h r G l u V a l T h r G l y C y s

-continued

1	5	10	15
Ala	Ala Asp Ile Ser Leu Gly Arg Ala Leu Ile Gln Ala Trp Gln Lys	20	25 30
Asp	Gly Ile Phe Gln Ile Lys Thr Asp Ser Glu Gln Asp Arg Lys Thr	35	40 45
Gln	Glu Ala Met Ala Ala Ser Lys Gln Phe Cys Lys Glu Pro Leu Thr	50	55 60
Phe	Lys Ser Ser Cys Val Ser Asp Leu Thr Tyr Ser Gly Tyr Val Ala	65	70 75 80
Ser	Gly Glu Glu Val Thr Ala Gly Lys Pro Asp Phe Pro Glu Ile Phe	85	90 95
Thr	Val Cys Lys Asp Leu Ser Val Gly Asp Gln Arg Val Lys Ala Gly	100	105 110
Trp	Pro Cys His Gly Pro Val Pro Trp Pro Asn Asn Thr Tyr Gln Lys	115	120 125
Ser	Met Lys Thr Phe Met Glu Glu Leu Gly Leu Ala Gly Glu Arg Leu	130	135 140
Leu	Lys Leu Thr Ala Leu Gly Phe Glu Leu Pro Ile Asn Thr Phe Thr	145	150 155 160
Asp	Leu Thr Arg Asp Gly Trp His His Met Arg Val Leu Arg Phe Pro	165	170 175
Pro	Gln Thr Ser Thr Leu Ser Arg Gly Ile Gly Ala His Thr Asp Tyr	180	185 190
Gly	Leu Leu Val Ile Ala Ala Gln Asp Asp Val Gly Gly Leu Tyr Ile	195	200 205
Arg	Pro Pro Val Glu Gly Glu Lys Arg Asn Arg Asn Trp Leu Pro Gly	210	215 220
Glu	Ser Ser Ala Gly Met Phe Glu His Asp Glu Pro Trp Thr Phe Val	225	230 235 240
Thr	Pro Thr Pro Gly Val Trp Thr Val Phe Pro Gly Asp Ile Leu Gln	245	250 255
Phe	Met Thr Gly Gly Gln Leu Leu Ser Thr Pro His Lys Val Lys Leu	260	265 270
Asn	Thr Arg Glu Arg Phe Ala Cys Ala Tyr Phe His Glu Pro Asn Phe	275	280 285
Glu	Ala Ser Ala Tyr Pro Leu Phe Glu Pro Ser Ala Asn Glu Arg Ile	290	295 300
His	Tyr Gly Glu His Phe Thr Asn Met Phe Met Arg Cys Tyr Pro Asp	305	310 315 320
Arg	Ile Thr Thr Gln Arg Ile Asn Lys Glu Asn Arg Leu Ala His Leu	325	330 335
Glu	Asp Leu Lys Lys Tyr Ser Asp Thr Arg Ala Thr Gly Ser	340	345 350

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1050 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGACCAACC TACAGACTTT CGAGTTGCCT ACCGAGGTAA CCGGCTGCGC CGCCGATATC

-continued

TCATTGGGAA	GGGCGCTGAT	CCAAGCCTGG	CAAAAAGATG	GCATTTTTC	GATCAAGACC	120
GATAGTGAGC	AGGATCGCAA	AACGCAGGAA	GCAATGGCTG	CTAGCAAGCA	GTTTTGCAAG	180
GAACCGCTGA	CTTTTAAGAG	TAGCTGCGTT	AGCGATCTGA	CCTACAGCGG	CTATGTTGCG	240
TCAGGCGAGG	AAGTCACAGC	TGGTAAACCT	GATTTCCCTG	AAATCTTCAC	TGTCTGCAAG	300
GACTTGTCGG	TAGGCGATCA	GCGTGTAATA	GCCGGCTGGC	CTTGCCATGG	TCCGGTGCCA	360
TGGCCAAATA	ACACCTATCA	GAAAAGCATG	AAGACCTTCA	TGGAAGAGCT	GGGTTTAGCG	420
GGCGAACGGT	TGCTCAAAC	GACAGCGCTC	GGCTTTGAAC	TACCCATCAA	CACGTTCCACC	480
GACTTAACTC	GCGATGGTTG	GCACCACATG	CGTGTATTAC	GCTTCCCGCC	CCAAACATCC	540
ACGCTGTCCC	GTGGAATTGG	TGCGCACACT	GACTATGGGT	TGTTGGTAAT	TGCCGCTCAG	600
GACGATGTTG	GTGGCTTATA	TATTCGCCCT	CCAGTCGAGG	GAGAGAAGCG	TAATCGTAAC	660
TGGTTGCCTG	GTGAGAGCTC	AGCAGGCATG	TTTGAGCACG	ATGAACCTTG	GACCTTCGTG	720
ACGCCACCC	CAGGCGTGTG	GACAGTTTTC	CCAGGTGATA	TCTTGCAGTT	CATGACCGGC	780
GGCCAGCTGC	TTTCCACTCC	GCACAAGGTT	AAGCTCAATA	CCCGCGAACG	TTTCGCCTGC	840
GCTTATTTTC	ATGAGCCTAA	TTTTGAAGCA	TCCGCCTATC	CGTTGTTTGA	GCCCAGCGCC	900
AATGAGCGTA	TTCATTATGG	TGAGCACTTT	ACCAACATGT	TTATGCGTTG	CTATCCAGAT	960
CGGATCACCA	CCCAGAGGAT	CAACAAGGAG	AATCGCCTGG	CGCACTTGGA	GGACTTGAAG	1020
AAGTATTCGG	ACACCCGCGC	GACAGGCTCA				1050

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1809 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCGACCGCGT	GTCAGCAACG	ACAATCCTTA	CTCGGAGTCG	CTGTTCAGGA	CACTGAAGTA	60
CTGCCCGCAA	TGGCCGCAGG	ATGGGTTTGC	CAGTCTTGAC	GCGGCACGCT	CTGGGTGAGG	120
GATTTTCATGC	GTTGGTACAA	CAATGATCAC	CGGCACAGCC	GAATCCGCTT	CGTGACACCG	180
GCTGAGCGGC	ATCGAGGGCT	GGATCATCAG	ATCCTGGCCA	AGCGACATGA	GCTGTACGAG	240
CTAGCCAGAG	AGAAAAGGCC	GGAGCGGTGG	TCGAGGGAGA	CACGCAACTG	GGAACCGATC	300
GGCACCGTGC	TGTAAACCC	GGATCGAGAG	CAAGACGTTG	AGAAAAAAGC	AGCATAGTTA	360
GACGGTTGAC	GCGACAATA	CCTTGAAAAA	CGCCGGCAGG	GACGCTCATG	ATTCATGCTC	420
CAAGCAGGTG	GGGTGTTTTT	CCGTCGCTTG	GACTTTGTTC	TCCCGACGTC	GTTTGGAAATG	480
AGCATCCGTC	CCTTTATATG	GATAAAGAAG	AGACTAGCAT	GACCAACCTA	CAGACTTTTCG	540
AGTTGCCTAC	CGAGGTAACC	GGCTGCGCCG	CCGATATCTC	ATTGGGAAGG	GCGCTGATCC	600
AAGCCTGGCA	AAAAGATGGC	ATTTTTTCAGA	TCAAGACCGA	TAGTGAGCAG	GATCGCAAAA	660
CGCAGGAAGC	AATGGCTGCT	AGCAAGCAGT	TTTGCAAGGA	ACCGCTGACT	TTTAAGAGTA	720
GCTGCGTTAG	CGATCTGACC	TACAGCGGCT	ATGTTGCGTC	AGGCGAGGAA	GTCACAGCTG	780
GTAAACCTGA	TTTCCCTGAA	ATCTTCACTG	TCTGCAAGGA	CTTGTCGGTA	GGCGATCAGC	840
GTGTAAAAGC	CGGCTGGCCT	TGCCATGGTC	CGGTGCCATG	GCCAAATAAC	ACCTATCAGA	900
AAAGCATGAA	GACCTTCATG	GAAGAGCTGG	GTTTAGCGGG	CGAACGGTTG	CTCAAACCTGA	960
CAGCGCTCGG	CTTTGAACTA	CCCATCAACA	CGTTCACCGA	CTTAACTCGC	GATGGTTGGC	1020

-continued

ACCACATGCG	TGTATTACGC	TTCCCGCCCC	AAACATCCAC	GCTGTCCCGT	GGAATTGGTG	1080
CGCACACTGA	CTATGGGTTG	TTGGTAATTG	CCGCTCAGGA	CGATGTTGGT	GGCTTATATA	1140
TTCGCCCTCC	AGTCGAGGGA	GAGAAGCGTA	ATCGTAACTG	GTTGCCTGGT	GAGAGCTCAG	1200
CAGGCATGTT	TGAGCACGAT	GAACCTTGGA	CCTTCGTGAC	GCCCACCCCA	GGCGTGTGGA	1260
CAGTTTTCCC	AGGTGATATC	TTGCAGTTCA	TGACCGGCGG	CCAGCTGCTT	TCCACTCCGC	1320
ACAAGGTAA	GCTCAATACC	CGCGAACGTT	TCGCCTGCGC	TTATTTTCAT	GAGCCTAATT	1380
TTGAAGCATC	CGCCTATCCG	TTGTTGAGC	CCAGCGCCAA	TGAGCGTATT	CATTATGGTG	1440
AGCACTTTAC	CAACATGTTT	ATGCGTTGCT	ATCCAGATCG	GATCACCACC	CAGAGGATCA	1500
ACAAGGAGAA	TCGCCTGGCG	CACTTGAGG	ACTTGAAGAA	GTATTCGGAC	ACCCGCGCGA	1560
CAGGCTCATG	AGTCGACACC	CTGCCCGGTG	CTGCCGGACA	GGGGCCTTAT	CGTTACTGGT	1620
GACTAATAAT	TGGCATATCA	ATGTCCACTC	AGCACCCAGA	TCTTATGATC	TGGGTGCTGA	1680
GTGGAGCAAT	GTAACCATTA	TGCTGAGCGT	TCATGCATAG	GAATTTCAAT	AATTCCTATA	1740
CAAGGCAATC	CGCCGAAAAA	GGTCCCCTCG	GCGTGATGCC	AACGTGGCGT	CGATGTCGGC	1800
AAAAAGCTT						1809

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1809 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: circular

(i i) MOLECULE TYPE: DNA (genomic)

- (i x) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 519..1568

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGACCGCGT	GTCAGCAACG	ACAATCCTTA	CTCGGAGTCG	CTGTTTCAGGA	CACTGAAGTA	60
CTGCCCGCAA	TGGCCGCAGG	ATGGGTTTGC	CAGTCTTGAC	GCGGCACGCT	CTGGGTGAGG	120
GATTTTCATGC	GTTGGTACAA	CAATGATCAC	CGGCACAGCC	GAATCCGCTT	CGTGACACCG	180
GCTGAGCGGC	ATCGAGGGCT	GGATCATCAG	ATCCTGGCCA	AGCGACATGA	GCTGTACGAG	240
CTAGCCAGAG	AGAAAAGGCC	GGAGCGGTGG	TCGAGGGAGA	CACGCAACTG	GGAACCGATC	300
GGCACCGTGC	TGTAAACCC	GGATCGAGAG	CAAGACGTTG	AGAAAAAAGC	AGCATAGTTA	360
GACGGTTGAC	GCGACAATA	CCTTGAAAAA	CGCCGGCAGG	GACGCTCATG	ATTCATGCTC	420
CAAGCAGGTG	GGGTGTTTTT	CCGTCGCTTG	GACTTTGTTC	TCCCGACGTC	GTTTGAATG	480
AGCATCCGTC	CCTTTATATG	GATAAAGAAG	AGACTAGC	ATG ACC AAC CTA CAG		533
				Met Thr Asn Leu Gln		
				1	5	
ACT TTC GAG TTG CCT ACC GAG GTA ACC GGC TGC GCC GCC GAT ATC TCA						581
Thr Phe Glu Leu Pro Thr Glu Val Thr Gly Cys Ala Ala Asp Ile Ser						
		10		15	20	
TTG GGA AGG GCG CTG ATC CAA GCC TGG CAA AAA GAT GGC ATT TTT CAG						629
Leu Gly Arg Ala Leu Ile Gln Ala Trp Gln Lys Asp Gly Ile Phe Gln						
		25		30	35	
ATC AAG ACC GAT AGT GAG CAG GAT CGC AAA ACG CAG GAA GCA ATG GCT						677
Ile Lys Thr Asp Ser Glu Gln Asp Arg Lys Thr Gln Glu Ala Met Ala						
		40		45	50	
GCT AGC AAG CAG TTT TGC AAG GAA CCG CTG ACT TTT AAG AGT AGC TGC						725
Ala Ser Lys Gln Phe Cys Lys Glu Pro Leu Thr Phe Lys Ser Ser Cys						

-continued

CGGCGTGATG CCAACGTGGC GTCGATGTCG GCAAAAAGCT T

1809

What is claimed is:

1. A DNA fragment comprising a gene encoding an ethylene-forming enzyme of bacteria represented by the following amino acid sequence:

SEQ ID NO:1:

Met Thr Asn Leu Gln Thr Phe Glu Leu Pro Thr Glu Val
 Thr Gly Cys Ala Ala Asp Ile Ser Leu Gly Arg Ala
 Leu Ile Gln Ala Trp Gln Lys Asp Gly Ile Phe Gln Ile
 Lys Thr Asp Ser Glu Gln Asp Arg Lys Thr Gln Glu
 Ala Met Ala Ala Ser Lys Gln Phe Cys Lys Glu Pro
 Leu Thr Phe Lys Ser Ser Cys Val Ser Asp Leu Thr
 Tyr Ser Gly Tyr Val Ala Ser Gly Glu Glu Val Thr Ala
 Gly Lys Pro Asp Phe Pro Glu Ile Phe Thr Val Cys Lys
 Asp Leu Ser Val Gly Asp Gln Arg Val Lys Ala Gly
 Trp Pro Cys His Gly Pro Val Pro Trp Pro Asn Asn
 Thr Tyr Gln Lys Ser Met Lys Thr Phe Met Glu Glu
 Leu Gly Leu Ala Gly Glu Arg Leu Leu Lys Leu Thr
 Ala Leu Gly Phe Glu Leu Pro Ile Asn Thr Phe Thr
 Asp Leu Thr Arg Asp Gly Trp His His Met Arg Val
 Leu Arg Phe Pro Pro Gln Thr Ser Thr Leu Ser Arg
 Gly Ile Gly Ala His Thr Asp Tyr Gly Leu Leu Val Ile
 Ala Ala Gln Asp Asp Val Gly Gly Leu Tyr Ile Arg Pro
 Pro Val Glu Gly Glu Lys Arg Asn Arg Asn Trp Leu
 Pro Gly Glu Ser Ser Ala Gly Met Phe Glu His Asp
 Glu Pro Trp Thr Phe Val Thr Pro Thr Pro Gly Val Trp
 Thr Val Phe Pro Gly Asp Ile Leu Gln Phe Met Thr
 Gly Gly Gln Leu Leu Ser Thr Pro His Lys Val Lys
 Leu Asn Thr Arg Glu Arg Phe Ala Cys Ala Tyr Phe
 His Glu Pro Asn Phe Glu Ala Ser Ala Tyr Pro Leu
 Phe Glu Pro Ser Ala Asn Glu Arg Ile His Tyr Gly Glu
 His Phe Thr Asn Met Phe Met Arg Cys Tyr Pro Asp
 Arg Ile Thr Thr Gln Arg Ile Asn Lys Glu Asn Arg Leu
 Ala His Leu Glu Asp Leu Lys Lys Tyr Ser Asp Thr
 Arg Ala Thr Gly Ser

2. A DNA fragment according to claim 1 which comprises a gene encoding an ethylene-forming enzyme of bacteria, wherein said bacteria belong to a genus *Pseudomonas*.

3. A DNA fragment according to claim 1 which comprises a gene encoding an ethylene-forming enzyme of bacteria represented by the following base sequence:

SEQ ID NO:2

ATG ACC AAC CTA CAG ACT TTC GAG TTG CCT
 ACC GAG GTA ACC GGC TGC GCC GCC GAT
 ATC TCA TTG GGA AGG GCG CTG ATC CAA

GCC TGG CAA AAA GAT GGC ATT TTT CAG
 ATC AAG ACC GAT AGT GAG CAG GAT CGC
 AAA ACG CAG GAA GCA ATG GCT GCT AGC
 AAG CAG TTT TGC AAG GAA CCG CTG ACT
 TTT AAG AGT AGC TGC GTT AGC GAT CTG
 ACC TAC AGC GGC TAT GTT GCG TCA GGC
 GAG GAA GTC ACA GCT GGT AAA CCT GAT
 TTC CCT GAA ATC TTC ACT GTC TGC AAG
 GAC TTG TCG GTA GGC GAT CAG CGT GTA
 AAA GCC GGC TGG CCT TGC CAT GGT CCG
 GTG CCA TGG CCA AAT AAC ACC TAT CAG
 AAA AGC ATG AAG ACC TTC ATG GAA GAG
 CTG GGT TTA GCG GGC GAA CGG TTG CTC
 AAA CTG ACA GCG CTC GGC TTT GAA CTA
 CCC ATC AAC ACG TTC ACC GAC TTA ACT
 CGC GAT GGT TGG CAC CAC ATG CGT GTA
 TTA CGC TTC CCG CCC CAA ACA TCC ACG
 CTG TCC CGT GGA ATT GGT GCG CAC ACT
 GAC TAT GGG TTG TTG GTA ATT GCC GCT
 CAG GAC GAT GTT GGT GGC TTA TAT ATT
 CGC CCT CCA GTC GAG GGA GAG AAG CGT
 AAT CGT AAC TGG TTG CCT GGT GAG AGC
 TCA GCA GGC ATG TTT GAG CAC GAT GAA
 CCT TGG ACC TTC GTG ACG CCC ACC CCA
 GGC GTG TGG ACA GTT TTC CCA GGT GAT
 ATC TTG CAG TTC ATG ACC GGC GGC CAG
 CTG CTT TCC ACT CCG CAC AAG GTT AAG
 CTC AAT ACC CGC GAA CGT TTC GCC TGC
 GCT TAT TTT CAT GAG CCT AAT TTT GAA
 GCA TCC GCC TAT CCG TTG TTC GAG CCC
 AGC GCC AAT GAG CGT ATT CAT TAT GGT
 GAG CAC TTT ACC AAC ATG TTT ATG CGT
 TGC TAT CCA GAT CGG ATC ACC ACC CAG
 AGG ATC AAC AAG GAG AAT CGC CTG GCG
 CAC TTG GAG GAC TTG AAG AAG TAT TCG
 GAC ACC CGC GCG ACA GGC TCA

4. A DNA fragment according to claim 1 which comprises a gene encoding an ethylene-forming enzyme of bacteria, wherein said fragment has a base sequence of at least from No. 519 to No. 1568 of the following base sequence:

SEQ ID NO:3:

10 20 30 40 50
 GCGACCGCGTGTCAGCAACGACAATCCTTACTCGGAGTCGCTGTTTCAGGACACTGAAGT
 60 70 80 90 100 110
 ACTGCCCGCAATGGCCGAGGATGGGTTTGCCAGTCTTGACGCGGCACGCTCTGGGTGAG
 120 130 140 150 160 170
 GGATTTTCATGCGTTGGTACAACAATGATCACCGGCACAGCCGAATCCGCTTCGTGACACC
 180 190 200 210 220 230
 GGCTGAGCGGCATCGAGGGCTGGATCATCAGATCCTGGCCAAGCGACATGAGCTGTACGA
 240 250 260 270 280 290
 GCTAGCCAGAGAGAAAAGGCCGGAGCGGTGGTTCGAGGGAGACACGCAACTGGGAACCGAT
 300 310 320 330 340 350
 CGGCACCGTGCTGTTAAACCCGGATCGAGAGCAAGACGTTGAGAAAAAAGCAGCATAGTT
 360 370 380 390 400 410
 AGACGGTTGACGCGACAACCTTGAAAAACGCCGGCAGGGACGCTCATGATTCATGCT

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420 430 440 450 460 470
 CCAAGCAGGTGGGGTGT TTTCCGTCGCTTGGACTTTGTTCTCCCGACGTCGTTTGG AAT
 480 490 500 510 520 530
 GAGCATCCGTCCCTTTATATGGATAAAGAAGAGACTAGCATGACCAACCTACAGACTTTC
 540 550 560 570 580 590
 GAGTTGCCTACCGAGGTAACCGGCTGCGCCGCCGATATCTCATTGGGAAGGGCGCTGATC
 600 610 620 630 640 650
 CAAGCCTGGCAAAAAGATGGCATT TTTTCAGATCAAGACCGATAGTGAGCAGGATCGCAAA
 660 670 680 690 700 710
 ACGCAGGAAGCAATGGCTGCTAGCAAGCAGTTT TGAAGGAACCGCTGACTTTTAAGAGT
 720 730 740 750 760 770
 AGCTGCGTTAGCGATCTGACCTACAGCGGCTATGTTGCGTCAGGCGAGGAAGT CACAGCT
 780 790 800 810 820 830
 GGTAAACCTGATTTCCCTGAAATCTTCACTGTCTGCAAGGACTTGTCCGGTAGGCGATCAG
 840 850 860 870 880 890
 CGTGTAAAAGCCGGCTGGCCTTGCCGTGGTCCGGTGCCATGGCCAAATAACACCTATCAG
 900 910 920 930 940 950
 AAAAGCATGAAGACCTTCATGGAAGAGCTGGGTTTAGCGGGCGAACGGTTGCTCAA ACTG
 960 970 980 990 1000 1010
 ACAGCGCTCGGCTTTGAACTACCCATCAACACGTT CACCGACTTAACTCGCGATGGTTGG
 1020 1030 1040 1050 1060 1070
 CACCACATGCGTGTATTACGCTTCCCGCCCCAAACATCCACGCTGTCCCGTGGAATTGGT
 1080 1090 1100 1110 1120 1130
 GCGCACACTGACTATGGGTTGTTGGTAATTGCCGCTCAGGACGATGTTGGTGGCTTATAT
 1140 1150 1160 1170 1180 1190
 ATTCGCCCTCCAGTCGAGGGAGAGAAGCGTAATCGTAACTGGTTGCCTGGTGAGAGCTCA
 1200 1210 1220 1230 1240 1250
 GCAGGCATGTTTGAGCACGATGAACCTTGGACCTTCGTGACGCCACCCCAGGCGTGTGG
 1260 1270 1280 1290 1300 1310
 ACAGTTTTCCAGGTGATATCTTGCAAGTTCATGACCGGCGGCCAGCTGCTTTCCACTCCG
 1320 1330 1340 1350 1360 1370
 CACAAGGTTAAGCTCAATACCCGCGAACGTTTCGCCTGCGCTTATTTTCATGAGCCTAAT
 1380 1390 1400 1410 1420 1430
 TTTGAAGCATCCGCCTATCCGTTGTTGAGGCCAGCGCCAATGAGCGTATTCATTATGGT
 1440 1450 1460 1470 1480 1490
 GAGCACTTTACCAACATGTTTATGCGTTGCTATCCAGATCGGATCACCACCCAGAGGATC
 1500 1510 1520 1530 1540 1550
 AACAAGGAGAATCGCCTGGCGCACTTGGAGGACTTGAAGAAGTATTCGGACACCCGCGCG
 1560 1570 1580 1590 1600 1610
 ACAGGCTCATGAGTCGACACCCTGCCCGGTGCTGCCGGACAGGGGCCTTATCGTTACTGG
 1620 1630 1640 1650 1660 1670
 TGACTAATAATTGGCATATCAATGTCCACTCAGCACCCAGATCTTATGATCTGGGTGCTG
 1680 1690 1700 1710 1720 1730
 AGTGGAGCAATGTAACCATTATGCTGAGCGTTCATGCATAGGAATTTCAATAATTCCTAT
 1740 1750 1760 1770 1780 1790
 ACAAGGCAATCCGCCGAAAAAGGTCCCCTCGGCGTGATGCCAACGTGGCGTCGATGTCCG
 1800
 CAAAAAGCTT

5. A vector which includes a DNA fragment which encodes the amino acid sequence defined in SEQ ID NO:1. 60

6. A host cell which has been transformed by the vector of claim 5.

7. The host cell of claim 6 wherein said host cell is *E. coli*.

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